Natural Deep Eutectic Solvents for functionalization and storage of natural products and enzymes

Baden, Camilla Knudsen; Montini, Lucia; Bassard, Jean-Étienne André; Viborg, Ketil Mathiasen; Jørgensen, Kirsten; Bjarnholt, Nanna; Møller, Birger Lindberg; Laursen, Tomas

Publication date:
2018

Document version
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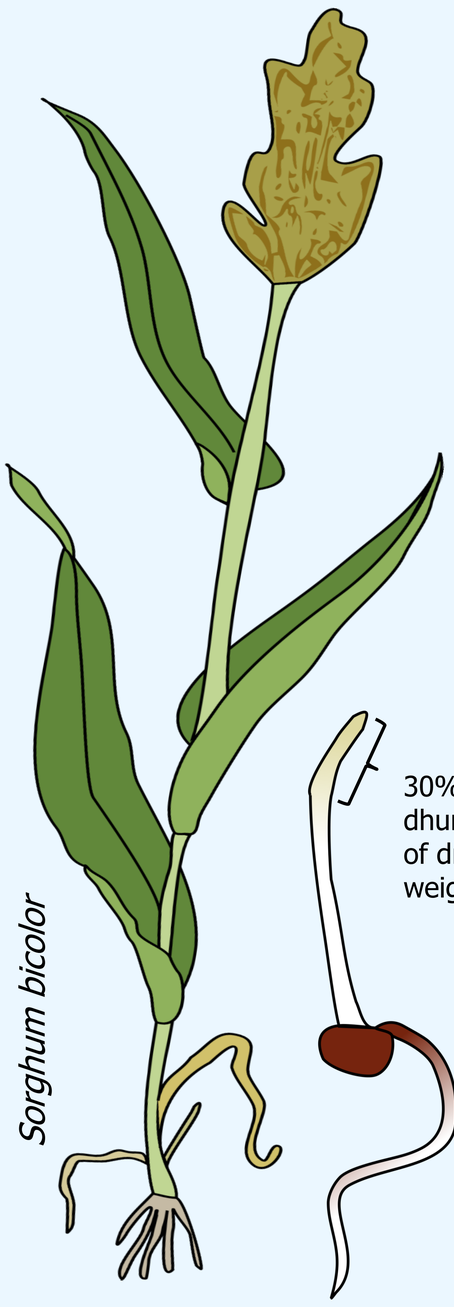
Camilla Knudsen, Lucia Montini, Jean-Etienne Bassard, Ketil Mathiasen Viborg (P-64) , Kirsten Jørgensen, Birger Lindberg Møller, Nanna Bjarnholt & Tomas Laursen (P-62)

camknud@plen.ku.dk



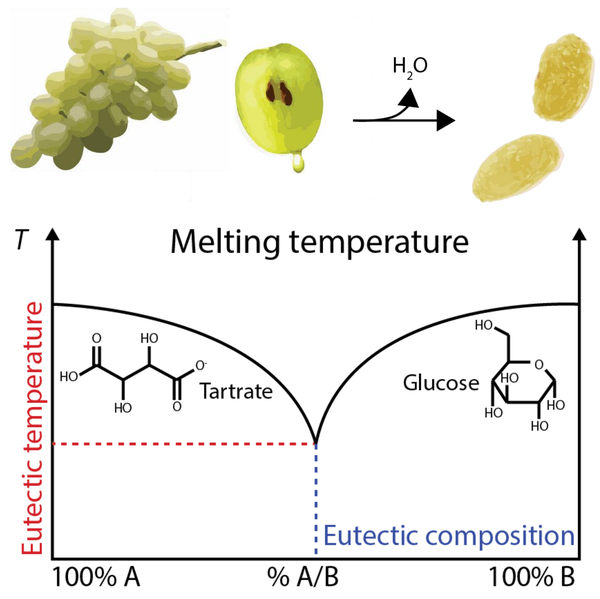
What are NAtural Deep Eutectic Solvents?

Biosynthesis and storage of natural products are organized by compartmentalization within the plant cell and offers the necessary plasticity for safe-storage of phytochemicals. Some natural products accumulate at intracellular concentrations above their solubility in water, up to molar concentrations.

Liquid-liquid phase separation of NAtural Deep Eutectic Solvents (NADESs) may facilitate the formation of dense biomolecular condensates within the cytosol or vacuole providing favorable environments facilitating this accumulation of natural products.

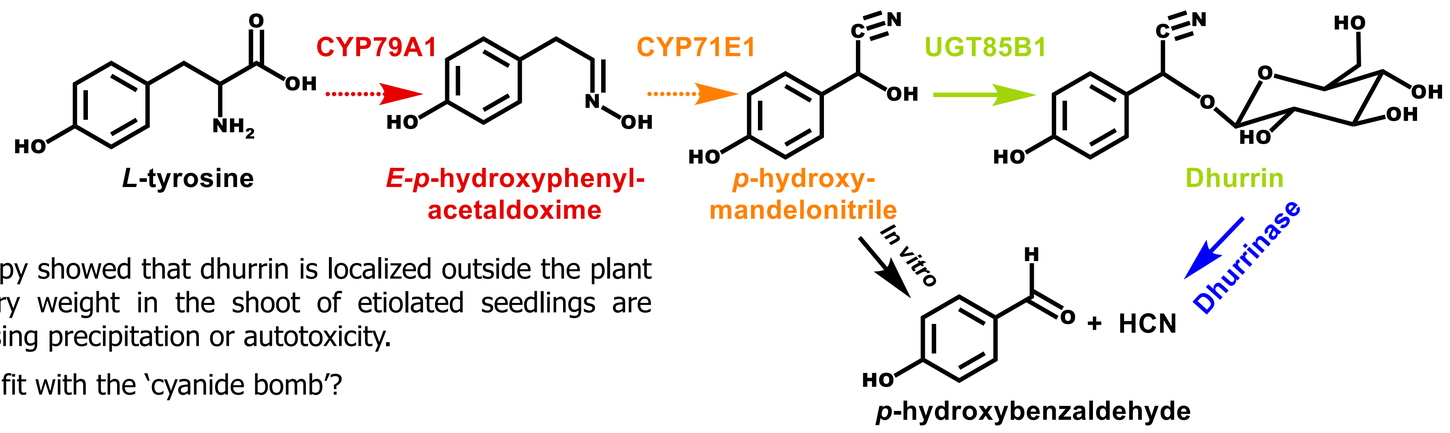
NADESs are composed of natural compounds including main plant cell metabolites such as sugars, amino acids, choline and organic acids (1). When mixed in equimolar ratios, these crystalline components may form a syrup-like liquid phase. This applies for a mixture of glucose:tartaric acid in a 1:1 molar ratio as found in raisins.

Raisins maintain a liquid phase despite an almost complete removal of water because glucose and tartaric acid interact through formation of intramolecular hydrogen bonds. This results in a high melting point depression causing the solids to liquefy and in many cases to remain fluid at room temperature (1).



Biosynthesis of the plant natural product dhurrin

Dhurrin is formed in the dhurrin metabolon (2), when activated by specific dhurrinase activity release of toxic HCN acts as a defense compound against chewing insects.

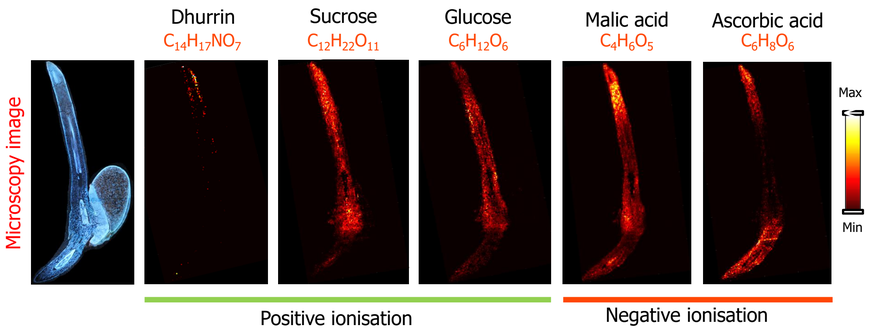


New study using Raman microscopy showed that dhurrin is localized outside the plant cell vacuole (3). 30% of the dry weight in the shoot of etiolated seedlings are composed of dhurrin without causing precipitation or autotoxicity.

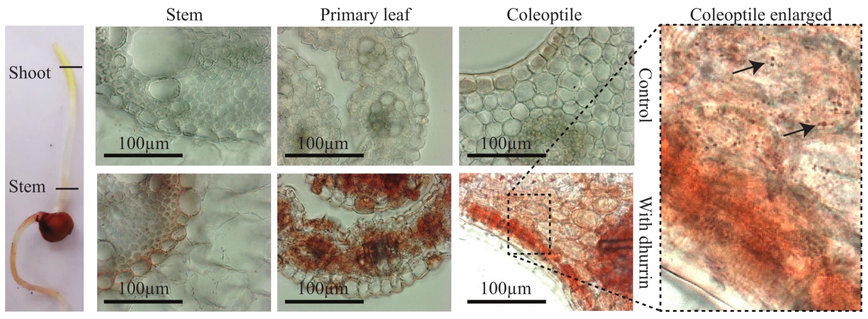
How does this fit with the 'cyanide bomb'?

Localization of dhurrin and dhurrinase

The biosynthesis and catabolism of dhurrin is tightly associated. The dhurrinases were isolated together with the dhurrin metabolon from etiolated seedlings (2).

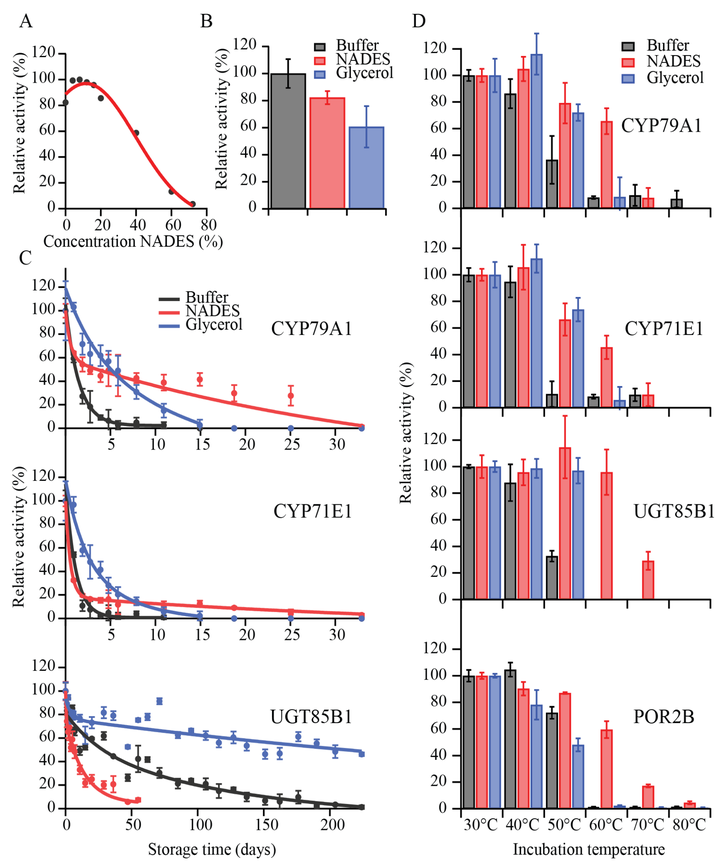


We used MALDI-MS-imaging (50µm resolution) to localize dhurrin, sugars and small organic acids, which could compose a NADES. Very high amounts of dhurrin in the edge of the shoot tip. Sugars and acids are more widely distributed.



Sugar reducing assay was used to detect dhurrinase activity in cross-sections of etiolated sorghum seedling, showing differences in activity between tissues that accumulate dhurrin. High activity in shoot and no activity in the stem. The enlargement show dhurrinase containing droplets in the cytosol in the coleoptile.

Functionalization of cytochromes P450 enzymes in NADESs



NADES may provide locale micro-environments around P450 metabolons facilitating metabolic channeling and preservation of the enzyme structure and activity during heat and drought stress in plants such as *Sorghum bicolor*.

CYP79A1, CYP71E1 and POR2b reconstituted into proteoliposomes prepared with sorghum lipids.

A) Optimal NADES concentration composed of tartrate and glucose (1:1 molar ratio) in activity assays were 12-15% , thus used for all assays.

B) UGT85B1 showed overall lower activity in NADES.

C) During long-term storage at RT, the UGT retained highest activity when store in glycerol. However the dhurrin CYPs showed improved activity when stored in NADES.

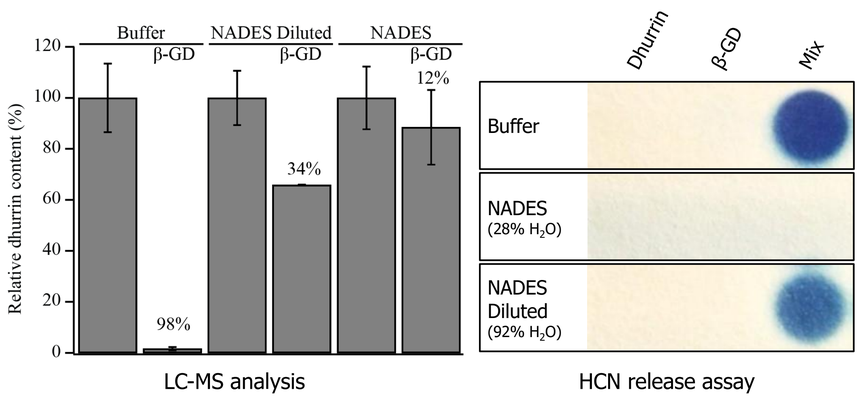
D) Similarly all dhurrin metabolon enzymes remained active after 30min heat treatment (30-60°C) in NADES outcompeting glycerol as storage medium.

NADES: An alternative compartmentalization phase

We demonstrate that droplets of NADES provide a previously unrecognized inert environment inside plant cells for co-storage of two-component defense systems like the 'cyanide bomb'.

Upon tissue disruption, e.g. by a chewing insect, dilution of the NADES leads to activation of the dhurrinase (β-GD) and ultimately release of toxic hydrogen cyanide.

The NADES droplets thus may function as an alternative to classical membrane-based compartmentalization and constitute a novel membrane-less compartment inside the plant cells.



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DEPARTMENT OF PLANT AND ENVIRONMENTAL SCIENCES
UNIVERSITY OF COPENHAGEN